Synergistic efficacy of botanical blends with and without synthetic insecticides against *Aedes aegypti* and *Culex annulirostris* mosquitoes

Essam Abdel-Salam Shaalan[1], Deon Vahid Canyon[2✉], Mohamed Wagdy Faried Younes[3], Hoda Abdel-Wahab[1], and Abdel-Hamid Mansour[1]

[1] *Zoology Department, Aswan Faculty of Science, South Valley University, Aswan, Egypt*
[2] *School of Public Health and Tropical Medicine, James Cook University, Townsville, Qld 4811, Australia*
[3] *Zoology Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt*

Received 22 February 2005; Accepted 26 July 2005

ABSTRACT: Increasing insecticide resistance requires strategies to prolong the use of highly effective vector control compounds. The use of combinations of insecticides with other insecticides and phytochemicals is one such strategy that is suitable for mosquito control. In bioassays with *Aedes aegypti* and *Culex annulirostris* mosquitoes, binary mixtures of phytochemicals with or without synthetic insecticides produced promising results when each was applied at a LC_{25} dose. All mixtures resulted in 100% mortality against *Cx. annulirostris* larvae within 24 h rather than the expected mortality of 50%. All mixtures acted synergistically against *Ae. aegypti* larvae within the first 24 h except for one mixture that showed an additive effect. We conclude that mixtures are more effective than insecticides or phytochemicals alone and that they enable a reduced dose to be applied for vector control potentially leading to improved resistance management and reduced costs. ***Journal of Vector Ecology* 30 (2): 284-288. 2005.**

Keyword Index: Botanical, phytochemical, insecticide, mosquito, synergism.

INTRODUCTION

Over the past decade, phytochemicals have received progressively more attention as insecticide alternatives. Roark (1947) described approximately 1,200 plant species that had been listed in the literature as having potential insecticidal value, while Sukumar et al. (1991) listed and discussed 344 plant species that only exhibited mosquitocidal activity. Most studies on the synergistic, antagonistic, and additive toxic effects of binary mixtures involving phytochemicals have been conducted on agricultural pests rather than vectors of diseases. The few studies on the mosquitocidal activity of binary mixtures have investigated the combined effects of phytochemicals with insecticides or microbial control agents. Synergism between synthetic insecticides and phytochemicals appears to be more common than among different phytochemicals, with some phytochemicals producing varied results depending on which synthetic insecticides they are mixed with. For instance, non-lethal concentrations of the volatile oil thymol and an unsaponifiable portion isolated from *Thymus capitatus* synergized the toxicity of malathion but induced additive or antagonistic effects when mixed with permethrin or pirimiphos-methyl insecticides in assays of *Culex pipiens* larvae (Mansour et al. 2000). This study thus aimed to identify alternative active botanical substances that could be combined with either existing synthetic mosquito control insecticides or other botanicals to produce synergistic or additive effects.

MATERIALS AND METHODS

Mosquito species and maintenance of cultures

Aedes aegypti were obtained from a colony initiated from mosquitoes collected in 2002 from Townsville, Australia. *Culex annulirostris* were bred from a colony maintained by the Queensland Institute of Medical Research in Brisbane, Australia. The colonies of mosquitoes were maintained at conditions of 27 ± 2 Cº and 70% ± 5 R.H. under 14L:10D cycles. *Ae. aegypti* larvae were kept in plastic buckets half filled with tap water and fed on goldfish flakes while *Cx. annulirostris* larvae were reared in aerated plastic trays half filled with dechlorinated tap water containing pieces of grass and fed on a mixture of granulated fish food (80%), liver powder (10%), and yeast (10%). Water in rearing containers was refreshed every 2 days. Adult mosquitoes were maintained on a 10% sugar solution while females were fed on rat blood.

Plant species and extraction

Seeds of *Khaya senegalensis* were collected from the Botanical Island in Aswan Province, Egypt. Seeds of *Daucus carota* were provided by Arthur Yates & Co Ltd (21A Richmond Road, Homebush, NSW 2140, Australia). Seeds were washed with running water, dried at room temperature, ground, bottled, and refrigerated until extraction. Essential oils of both *K. senegalensis* and *D. carota* seeds were extracted according to the method of Stein and Klingauf (1990). A quantity of ground seed (5 - 15 g) was extracted for 4 h with 150-300 ml of solvent (acetone, ethanol, hexane, and methanol) in a Soxhlet apparatus. The different crude extracts, comprised primarily of essential oils, were separated under vacuum by using a rotary evaporator at temperatures equivalent to the boiling points of the solvents used.

The three *Callitris* extracts were supplied by Michael Kennedy, Queensland Forestry Research Institute, Department of Primary Industries, Queensland Government, Australia. Steam volatile oil was obtained by distillation from cypress sawdust in water at atmospheric pressure. In this method, the most volatile components of the wood float on the condensate water as an oil. The second extract from cypress sawdust was obtained by using liquefied refrigerant gas (i.e., under pressure), followed by removal of the refrigerant by flashing off by reducing the pressure. This extract contained a balance of more volatile and less volatile components. The third extract from cypress sawdust was obtained using methanol under reflux followed by removal of methanol by distillation, further removal under vacuum (rotary evaporator), and freeze drying of the remainder. Much of the more volatile components were lost in this process. These extracts, produced in early 2004, were sealed, refrigerated, and protected from light until use.

Synthetic Insecticides and growth regulators

Technical grade organophosphorous and pyrethroid insecticides (fenitrothion 96.8% and lambda-cyhalothrin 90.99%) were provided by Nufarm Ltd (103-105 Pipe Road, Laverton, 3026 North Victoria, Australia). Technical grade (s)-methoprene, an insect growth regulator, was obtained from Wellmark International (1100 East Woodfield Road, Suite 500, Schaumburg, IL 60173, U.S.A.).

Bioassay

Two groups of mixtures were evaluated against both *Ae. aegypti* and *Cx. annulirostris* larvae. The first group contained mixtures from botanical extracts and synthetic insecticides, while the second group contained mixtures from botanical extracts only. The mixtures were prepared according to the method described by Moawed[4]. All mixtures consisted of a 1:1 (v/v) ratio of the LC_{25} dose of each compound. Four ml from each test substance were mixed together in a glass vial. Two ml of the mixture were placed in a glass beaker containing 98 ml de-ionized water and 25 newly-emerged 4th instar mosquito larvae. Each test was replicated four times with one control and mortality was recorded daily till the death of all larvae or adult emergence. The equation below (Sun and Johnson 1960) was used to evaluate the joint effect of the different binary mixtures after 24 h.

$$\text{Co-toxicity factor} = \frac{\text{observed \% mortality} - \text{expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

This factor differentiates the results into three categories. A positive factor of ≥ 20 indicates potentiation, a negative factor of ≤ -20 indicates antagonism, and the intermediate values of >-20 to < 20 indicate an additive effect. Because obtained LC_{25}s are mathematically estimated, they were tested again against mosquito larvae to determine the expected mortality. The expected mortality of the combined pair is the sum of the mortalities of single compound at the given concentration LC_{25}. The observed mortality is the recorded mortality obtained 24 h after using the mixtures.

RESULTS

All mixtures tested against *Ae. aegypti* larvae showed synergistic effects except a mixture of *K. senegalensis* hexane extract and steam distilled *C. glaucophylla* which showed an additive effect (Table 1). All mixtures tested against *Cx.*

[4]Moawed, H.A.M. 1998. Joint action of some plant extracts against the mosquito larvae of *Culex pipiens* and their physiological impact. M.Sc. Thesis, Faculty of Science-Dmietta, Mansoura University, Egypt.

Table 1. Effect of mixtures from insecticides and botanical extracts at 1:1 ratios (LC_{25} + LC_{25}) against newly-molted 4th instar *Aedes aegypti* larvae.

Insecticides and botanical extracts		*Callitris glaucophylla* extracts									*Khaya senegalensis*		
		Steam distillation			Liquefied refrigerant gas			Methanol reflux			(Hexane extract)		
		Joint action	Adult emergence (%)		Joint action	Adult emergence (%)		Joint action	Adult emergence (%)		Joint action	Adult emergence (%)	
			Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.
Insecticides	Fenitrothion	S	1	93	S	0	93	S	0	96	S	2	96
	Lambda-cyhalothrin	S	1	99	S	1	96	S	0	93	S	3	99
	Methoprene	S	0	93	S	0	93	S	0	93	S	0	99
Callitris glaucophylla	Steam distillation extract				S	0	96	S	0	99	A	0	96
	Liquefied refrigerant gas extract							S	0	99	S	0	96
	Methanol reflux extract										S	0	99

S = synergism. A = additive.

Table 2. Effect of mixtures from insecticides and botanical extracts at 1:1 ratios ($LC_{25} + LC_{25}$) against newly-molted 4th instar *Culex annulirostris* larvae.

Botanical extracts		Insecticides					
		Fenitrothion			Lambda-cyhalothrin		
		Joint action	Adult emergence (%)	L. M. in Cont.	Joint action	Adult emergence (%)	L. M. in Cont.
Callitris glaucophylla	Steam distillation extract	S	0	1	S	0	1
	Liquefied refrigerant gas extract	S	0	7	S	0	4
	Methanol reflux extract	S	0	7	S	0	7
Daucus carota	Acetone extract	S	0	3.7*	S	0	6.5*
	Ethanol extract	S	0	5.6*	S	0	5.6*
	Hexane extract	S	0	2	S	0	6.7*
	Methanol extract	S	0	2	S	0	2.5*
Khaya senegalensis	Acetone extract	S	0	1	S	0	1
	Ethanol extract	S	0	1	S	0	1
	Hexane extract	S	0	1	S	0	1
	Methanol extract	S	0	1	S	0	1

L. M. in Cont. = larval mortality % in control. S = synergism. * Emerged pupae not included in calculations.

annulirostris larvae showed synergistic effects and caused 100% larval mortality within the first 24 h (Tables 2 and 3).

Out of 18 mixtures tested, five did not completely inhibit adult *Ae. aegypti* emergence. Mixtures of fenitrothion with steam-distilled *C. glaucophylla* and a *K. senegalensis* hexane extract resulted in 1 and 2% emergence, respectively. Lambda-cyhalothrin mixed with steam-distilled and liquefied refrigerant gas extracts of *C. glaucophylla* and a *K. senegalensis* hexane extract led to 1, 1, and 3% emergence, respectively. Both groups of mixtures, botanical extracts with or without insecticides, were observed to completely inhibit adult *Cx. annulirostris* emergence within the first 24 h.

While both mixtures of botanicals and mixtures of botanicals with methoprene effectively inhibited adult *Ae. aegypti* emergence, mixtures of insecticides with botanicals did not completely inhibit emergence although the emergence rate of 1–3% was negligible compared with 93–99% in untreated controls.

All binary mixtures proved to be more effective than all other non-mixed sublethal concentrations (LC_{25}, LC_{50}, and LC_{75}) of both insecticide and phytochemical, and were similar in efficacy to non-mixed LC_{100} doses.

DISCUSSION

The synergistic effects observed in bioassays using a combination of botanical extracts and different synthetic insecticides have been observed in several previous studies (Kalyanasundaram and Babu 1982, Kalyanasundaram and Das 1985, Mulla and Su 1999, Thangam and Kathiresan 1990, 1991). Some extracts have also produced synergistic effects with insect growth regulators. Mulla and Su (1999) showed that neem seed kernel extract has synergistic effects when combined with the juvenile hormone analog methoprene. A few studies have mentioned synergism between different botanical extracts. Mwaiko (1992) reported that a mixture of the peel oils extract of three citrus species (lemon, orange, and bitter orange) was much more effective than for the peel oils extract for the individual species. Moawed (1998) studied the joint action of binary mixtures of some plant extracts with each other and with the synthetic pyrethroid insecticide cypermethrin against *Cx. pipiens* larvae.

In addition to response variations caused by plant species, extraction method, and extraction solvent, the results from this study show that insecticidal efficacy of binary mixtures from botanical extracts with or without synthetic insecticides against mosquitoes varies based on mosquito genera. This difference in efficacy against both mosquito genera could be attributed to a difference in physiological response among mosquito genera and species and not to the effect of the mixtures. Surprisingly, it is the first study to mention the effect of mosquito genera on the efficacy of insecticide mixtures. The mechanism of synergism is not known, but Thangam and Kathiresan (1991) stated that synergism might be due to phytochemicals inhibiting the ability of mosquito larvae to employ detoxifying enzymes against synthetic chemicals. Mixtures may be useful in prolonging the lifetime of various cost-effective synthetic insecticides providing that care is taken not to use botanicals that are affected by the same resistance mechanisms that target the synthetic insecticide. Further cost benefits are possible due to increased potency despite the use of lower concentrations. When this is combined

Table 3. Effect of mixtures from botanical extracts at 1:1 ratios ($LC_{25} + LC_{25}$) against newly-molted 4th instar *Culex annulirostris* larvae.

Botanical extracts		*Callitris glaucophylla*									*Daucus carota*									*Khaya senegalensis*								
		Steam distillation			Liquefied refrigerant			Methanol reflux			Ethanol			Hexane			Methanol			Ethanol			Hexane			Methanol		
		JA	AE	MC	JA	AE	MC	JA	AE	MC	JA	AE	MC	JA	AE	MC	JA	AE	MC	JA	AE	MC	JA	AE	MC	JA	AE	MC
Callitris glaucophylla	Steam distillation				S	0	5	S	0	5	S	0																
	Liquefied refrigerant gas							S	0	7	S	0																
	Methanol reflux										S	0																
Daucus carota	Acetone	S	0	5	S	0	5	S	0	4	S	0	7	S	0	1	S	0	5	S	0	1	S	0	1	S	0	1
	Ethanol	S	0	3	S	0	6	S	0	4				S	0	3	S	0	6									
	Hexane	S	0	2	S	0	6	S	0	4							S	0	3									
	Methanol	S	0	2	S	0	3	S	0	4																		
Khaya senegalensis	Acetone	S	0	1	S	0	6	S	0	1	S	0	6	S	0	6	S	0	3	S	0	1	S	0	5	S	0	5
	Ethanol	S	0	4	S	0	5	S	0	4	S	0	6	S	0	2	S	0	3				S	0	6			
	Hexane	S	0	4	S	0	5	S	0	5	S	0	3	S	0	7	S	0	3				S	0	5			
	Methanol	S	0	5	S	0	4	S	0	4	S	0	6	S	0	2	S	0	2	S	0	5	S	0				

JA = joint action. AE = adult emergence. MC = percentage larval mortality in control. S = synergism.

with the degree of emergence inhibition observed and the long-lasting effects (Chockalingam et al. 1990), the benefits of synergistic mixtures are clear. The identification of botanical compounds that prove to be highly synergistic when combined with currently used synthetic insecticides will lead to more cost-effective mosquito control and have the potential to minimize the development of resistance caused by frequent application of mono insecticides.

Acknowledgments

We are grateful to Dr Michael Kennedy, Queensland Forestry Research Institute, Department of Primary Industries, Queensland Government, Australia, for providing us with extracts of *C. glaucophylla*. We are also grateful to Dr Wayne Melrose, School of Public Health and Tropical Medicine, James Cook University, Queensland, Australia, for funding solvents required in this work. We are appreciative of Professor Bruce Bowden, School of Pharmacy and Molecular Sciences, James Cook University, Queensland, Australia, for his help, guidance, and unlimited facilities for conducting phytochemical extraction in his laboratory.

REFERENCES CITED

Chockalingam, S., S. Thenmozhi, and M.S.N. Sundari. 1990. Larvicidal activity of different products against mosquito larvae. J. Environ. Biol. 11: 101-104.

Kalyanasundaram, M. and C.J. Babu. 1982. Biologically active plant extracts as mosquito larvicides. Ind. J. Med. Res. 76: 102-106.

Kalyanasundaram, M. and P. K. Das. 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Ind. J. Med. Res. 82: 19-23.

Mansour, S.A., S.S. Messeha, and S.E. EL-Gengaihi. 2000. Botanical biocides. 4. Mosquitocidal activity of certain *Thymus capitatus* constituents. J. Nat. Tox. 9: 49-62.

Mulla, M.S. and T. Su. 1999. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J. Am. Mosq. Contr. Assoc. 15: 133-152.

Mwaiko, G.L. 1992. Citrus peel oil extracts as mosquito larvae insecticides. E. Afr. Med. J. 69: 223-226.

Roark, R.C. 1947. Some promising insecticidal plants. Econ. Bot., 1: 437. Cited in: C. F. Curtis, (ed.) *Appropriate Technology in Vector Control*. CRS Press, Florida, 1990.

Stein, U. and F. Klingauf. 1990. Insecticidal effect of plant extracts from tropical and subtropical species. J. Appl. Entomol. 110: 160-166.

Sukumar, K., M.J. Perich, and L.R. Boobar. 1991. Botanical derivatives in mosquito control: A review. J. Am. Mosq. Contr. Assoc. 7: 210-237.

Sun, Y.P. and E.R. Johnson. 1960. Analysis of joint action of insecticides against house flies. J. Econ. Entomol. 53: 887-892.

Thangam, T.S. and K. Kathiresan. 1990. Synergistic effects of insecticides with plant extracts on mosquito larvae. Trop. Biomed. 7: 135-137.

Thangam, T.S. and K. Kathiresan. 1991. Mosquito larvicidal activity of marine plant extracts with insecticides. Botanica Marina 34: 537-539.